## DRUG FORMULATIONS AND CLINICAL METHODS

# Analytical Eco-Scale for Assessing the Greenness of a Developed Potentiometric Method for Lomefloxacin Hydrochloride Determination in its Different Dosage Forms, Plasma, and Dissolution Medium

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**Background:** Traditional methods for Lomefloxacin hydrochloride (LOM) determination involve pretreatment steps, which extend analysis time and use hazardous chemicals. **Objective:** The ability to provide a rapid route without sample pretreatment for quantitative determination of compounds via a low-cost instrument is a challenging task. In this work, a simple potentiometric method was developed to determine the antibacterial LOM via in-house fabricated ion selective electrodes. **Methods:** Different sensors were fabricated using a poly vinyl chloride-based membrane, potassium tetrakis(4-chlorophenyl) borate as a cation exchanger, and 2-Nitrophenyl octyl ether as a plasticizer (sensor 1). To increase the selectivity of sensor 1, a selective molecular recognition component 2-hydroxypropyl-β-cyclodextrin was used as ionophore (sensor 2). **Results:** The proposed method was validated according to International Union of Pure and Applied Chemistry recommendations, in which the proposed sensors show a linear dynamic range from $1 \times 10^{-5}$ to $1 \times 10^{-2}$ mol/L, with Nernstian slopes of 55.829 and 58.229 mV/decade for sensors 1 and 2, respectively. It was applied to determine LOM in bulk powder, in different dosage forms, and in plasma with no sample pretreatment. Also, the suggested method can be used as a green, in-line bench top real-time analyzer for in-process monitoring of LOM release from its tablets, under U.S. Food and Drug Administration dissolution regulations, with clear discrimination from common excipients. Results obtained by the proposed potentiometric method were compared with those obtained by a reported HPLC method. **Conclusions:** The proposed method is considered as a perfect alternative to traditional reported methods for LOM determination.

Direct testing without sample pretreatment is the most environmentally friendly method of analysis. Traditional analysis methods involve extraction or purification steps, which lengthen the analysis time and also use many different hazardous organic solvents. Applying a direct method such as potentiometry is considered the best alternative for traditional methods. Potentiometric methods are one of the most environmentally friendly methods of analysis (1), in addition to their applicability over a wide range of concentrations that can extend over many orders of magnitude.

Dissolution test is the applied technique to detect pharmaceutical formulation problems and to correlate in vitro and in vivo studies (2). Drug determination in dissolution experiments are primarily carried out by traditional spectrophotometric or HPLC methods. Samples are frequently withdrawn to be analyzed at different time intervals, which is a tedious time-consuming task, along with pretreatment procedures. The automation in the dissolution systems was introduced but with several disadvantages either in UV detection such as turbidity or in HPLC methods such as discontinuous profiles. The automation terminates the process of sample withdrawal at different time intervals. However, it requires expensive setup, additional analysis time, and solvent consumption. Therefore, development of a rapid in-line analytical method, which is continuous and not affected by colored and colloidal systems, is a perfect idea to determine the drug concentration during dissolution testing.

Lomefloxacin hydrochloride (LOM; Figure 1) is chemically 1-ethyl-6,8-difluoro-7-(3-methylpiperazin-1-yl)-4-oxo-1, 4-dihydroquinoline-3-carboxylicacid hydrochloride (3). LOM is a fluoroquinolone antibiotic used to treat bacterial infections, including bronchitis, eye infections, and urinary tract infections. It is also used to prevent urinary tract infections prior to surgery (3). LOM is formulated as tablets and eye drops.

The literature review reveled that LOM was determined previously by spectrophotometric methods (4–6), HPLC methods (7–20), and electrochemical methods (21, 22). Some of the reported methods were applied to determine LOM in pharmaceutical formulations (4–10, 17, 22). Others were used to determine LOM in biological fluids, but all involve extraction and purification steps (11–16, 18–20).

The aim of this work was to develop ion-selective electrodes for determination of LOM without prior separation from its formulation matrix both in tablets or eye drops as well as in human plasma. Furthermore, this work aimed to apply the fabricated electrodes for in-line monitoring of the dissolution of LOM from its tablets and to compare the obtained results with those obtained by applying a traditional HPLC method.
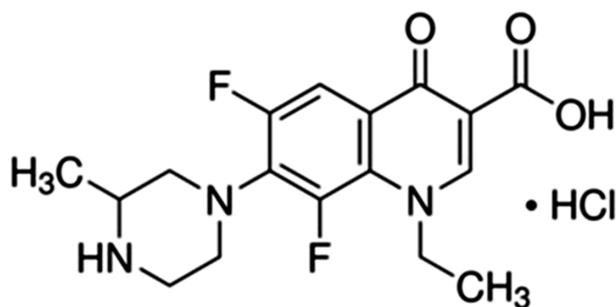
**Figure 1. Chemical structure of lomefloxacin hydrochloride.**

## Experimental

### Apparatus

(**a**) *Jenway digital ion analyzer*.—Model 3330 (Essex, United Kingdom).

(**b**) *Ag⁰/AgCl double junction reference electrode*.—Orion 900200 (Thermo Fisher Scientific, Waltham, MA); 3.0 M KCl saturated with AgCl as an inner filling solution and 10% KNO₃ as a bridge electrolyte.

(**c**) *pH glass electrode*.—Jenway (UK) No. 924005-BO3-Q11C.

(**d**) *Magnetic stirrer*.—Bandelin Sonorox, Rx510S (Budapest, Hungary).

(**e**) *VanKel VK 7000 USP II (Paddle) apparatus*.—Used to perform the dissolution. It consists of six vessels each containing 900 mL of 0.01 N HCl, thermostatically set at $37\pm0.5°C$. The medium was agitated using a paddle at a rotation rate of 50 rpm.

(**f**) *HPLC system*.—Agilent 1260 Infinity series, operated by Agilent Chemstation software. A quaternary pump injector with a 20 μL loop and a diode array detector (Minnetonka, MN) were used.

(**g**) *A Kinetex 5 μm Evo C18 RP column*.—250 × 4.6 mm using a mixture of acetonitrile–mixed phosphate buffer pH 4 (45 + 55, v/v) at 1 mL/min as a mobile phase.

### Chemicals and Reagents

(**a**) *Polyvinylchloride (PVC) of high molecular weight, sodium tetraphenyl borate (TPB), potassium tetrakis (4-chlorophenyl) borate (KTCPB), 2-nitrophenyloctylether (NPOE), 2-hydroxypropyl-β-cyclodextrin (2-HP βCD), tetrahydrofuran (THF), acetonitrile HPLC grade*.—Obtained from Sigma Aldrich, Germany.

(**b**) *Potassium chloride, hydrochloric acid, disodium hydrogen orthophosphate, and potassium dihydrogen orthophosphate*.—Obtained from El-Nasr Pharmaceuticals, Cairo, Egypt.

(**c**) *Human plasma*.—Obtained from El-Kasr El-Aini blood bank, Egypt.

(**d**) *Deionized water*.—Obtained from an Elga Ultrapure Q apparatus.

(**e**) *LOM working standard*.—Supplied by Future Pharmaceutical Industries, Giza, Egypt, and its purity was certified to be 99.3%.

(**f**) *Lomeflox tablets*.—Labeled to contain 441.5 mg lomefloxacin hydrochloride equivalent to 400 mg lomefloxacin, Batch No. 160931A) were used. The tablets were manufactured by Future Pharmaceutical Industries for Internal Trading Office, Giza, Egypt, and were purchased from the local market.

(**g**) *Orchacin eye drops*.—Labeled to contain 3.31 mg lomefloxacin hydrochloride equivalent to 3 mg lomefloxacin, Batch No. 0515163) manufactured by Orchidia Pharmaceutical Industries, Cairo, Egypt, were purchased from the local market.

### Standard Solutions

LOM stock standard solution $(1.0\times10^{-2}$ mol/L) was prepared in 0.01 N HCl. Working standard solutions $(1.0\times10^{-7}$ to $1.0\times10^{-2}$ mol/L) were prepared by suitable dilution of the stock solution using 0.01 N HCl.

### Procedure

(**a**) *Membrane fabrication and sensors assemblies preparation*.—In a two 5 cm diameter glass Petri dish, 0.4 mL of NPOE was mixed with 190 mg PVC and 10 mg KTCPB (sensor I), and 0.4 mL of NPOE was mixed with 190 mg PVC, 10 mg KTCPB, and 10 mg of 2-HP βCD (sensor II). The mixtures were dissolved in 6 and 8 mL of THF for sensors I and II, respectively. Each Petri dish was then covered with a Whatman No. 3 filter paper and left to stand overnight at room temperature to allow solvent evaporation. A master membrane with a thickness of 0.1 mm was obtained. From each master membrane, a disk approximately 7 mm diameter was cut and then fixed using THF to an elastic PVC tip, which was fixed into the end of a glass electrode body. Equal volumes of $1\times10^{-3}$ mol/L LOM and $1\times10^{-3}$ mol/L potassium chloride (both prepared in 0.01 N HCl) were mixed and used as an internal reference solution. Ag⁰/AgCl wire (1 mm diameter) was used as an internal reference electrode when immersed in the internal reference solution. The sensors were conditioned by soaking in $1\times10^{-3}$ mol/L LOM standard solution for 24 h and stored in the same solution when not in use.

(**b**) *Sensors calibration*.—Calibration was done in a series of 25 mL beakers by transferring aliquots of LOM working solutions $(1.0\times10^{-7}$ to $1.0\times10^{-2}$ mol/L). Each prepared electrode was immersed in conjunction with a double junction Ag⁰/AgCl reference electrode in each solution, and the potential was measured. The electrode was washed with 0.01 N HCl between measurements. The developed potentials were plotted versus log-arithmic concentration of LOM. The regression equations were computed and used for measurement of unknown samples. The electrochemical performance of sensor I and sensor II was evaluated according to International Union of Pure and Applied Chemistry (IUPAC) recommendations (23).

(**c**) *Effect of different pH on electrode response*.—Effect of changing pH on the potential values in the range from 1 to 12 was investigated by using 2N solution of NaOH and 2N solution of HCl to adjust the pH of a $10^{-3}$ and $10^{-4}$ mol/L LOM solution. The potential obtained at each pH value was recorded.

(**d**) *Sensor selectivity*.—Potentiometric selectivity coefficient $(K^{pot}_{LOM, interferent})$ was calculated to estimate the degree to which an interfering substance or excipient would interfere with the response of the electrodes to its primary ion. The selectivity coefficients were evaluated according to IUPAC guidelines using the separate solutions method (23), employing the following equation:

$$-\log\left(K^{pot}_{primary\ ion,interferent}\right) = (E1 - E2)/S$$

where $E1$ is the potential measured (millivolts) in $1.0 \times 10^{-3}$ mol/L of LOM solution; $E2$ is the potential measured in $1.0 \times 10^{-3}$ mol/L of the interfering substance solution; and $S$ represents the slope of the proposed sensor (millivolt/concentration decade).

(**e**) *Potentiometric determination of laboratory-prepared mixtures containing different ratios of LOM and benzalkonium chloride (BZCL).*—In a series of 25 mL measuring flasks, 2.5 mL of $1 \times 10^{-2}$ mol/L LOM were mixed with different concentrations of BZCL around the concentration of BZCL in Orchacin eye drops (50, 100, 200, 300, and 400%). The potential of the prepared mixtures was recorded using the proposed sensors coupled with the reference electrode, and the concentration of LOM was determined using the corresponding regression equation.

(**f**) *Potentiometric determination of LOM in its pharmaceutical formulations.*—(*1*) *Lomeflox tablets.*—Ten tablets were weighed and then finely powdered. An accurately weighed portion of the powder claimed to contain $1 \times 10^{-3}$ mol/L LOM was transferred to a 50 mL measuring flask and completed to the mark with 0.01 N HCl. The potential was recorded, and LOM concentration was determined using the corresponding regression equation.

(*2*) *Orchacin eye drops.*—An accurate volume of the eye drop solution claimed to contain $1 \times 10^{-3}$ mol/L LOM was transferred to 25 mL measuring flask and completed to the mark with 0.01 N HCl. The potential was recorded, and LOM concentration was determined using the corresponding regression equation.

(**g**) *Potentiometric determination of LOM in plasma.*—In three 25 mL measuring flasks each containing 1 mL plasma, 2.5 mL of $10^{-2}$, $10^{-3}$, and $10^{-4}$ mol/L LOM solution were added separately, and the volume was completed with 0.01 N HCl. The potentials were measured by the proposed sensors, and LOM concentration was determined from the corresponding regression equation. Sensor washing was performed between measurements using 0.01 N HCl.

(**h**) *Potentiometric determination of percent dissolution of LOM from LOM tablets.*—The reference electrode jointly with the investigated sensor was introduced in the dissolution vessel. The potentiometric readings were recorded at 0, 5, 8, 10, 15, 20, 25, 30, 40, 45 min and converted to percentage dissolution using the transpose of the Nikolskii–Eisenman equation, as follows (24):

$$C_{analyte} = C_{st}\left(10^{E/S} - 1\right)$$

where $C_{analyte}$ is the analyte concentration; $C_{st}$ is a constant; $E$ is the potential in millivolts; and $S$ is the slope of the investigated sensor. The dissolution profile was obtained relating the percent dissolution to the time.

(**i**) *Determination of percent dissolution of LOM from LOM tablets by HPLC method (8).*—At different time intervals of 0, 10, 20, 30, 45 min, samples were withdrawn from the dissolution vessel, filtered, diluted with 0.01 N HCl, and injected into the HPLC system. Peak areas were recorded at 254 nm and used to obtain the corresponding concentration of LOM. The percentage dissolution was calculated, and the dissolution profile was drawn relating the percent dissolution to the time.

## Results and Discussion

### Characteristics of Ion-Selective Electrodes

The performance of ion-selective electrodes depends on the nature and lipophilicity of the ion exchanger (25), the solvent mediator (26), and other additives such as different ionophores (27).

The main rule of the ion exchanger is to make the membrane perm-selective to the drug ion (25). LOM behaves as a cation because of the presence of a basic amino group in its structure; this suggests the use of a cation exchanger to form the ion pair in the LOM sensor. The type of the exchanger affects the sensor response; therefore, two cation exchangers were tried, namely TPB or KTCPB. The sensors were tested for their responses toward LOM concentration; TPB-based sensors do not produce good response to LOM concentration compared with the linear response obtained with KTCPB-based sensor. Therefore, sensor I was prepared using KTCPB as an ion exchanger. The ratio of the ion exchanger to LOM in the formed ion pair is 1:1, as proven by the obtained Nernstian slopes (around 60 mV/decade), so LOM acts as a monoionic species. Previously, sensors were prepared by incorporating the ion pair complex during the preparation of the membrane (22). In our study, the ion pairs were formed in situ by soaking the fabricated membranes in $1 \times 10^{-3}$ mol/L LOM solution for 1 day to replace the original exchangeable counter ion ($K^+$) of the ion exchanger with LOM, so the membrane potential becomes responsive to the drug concentration. Ion pair formation by soaking the plain membrane in LOM solution is more economic and enables the versatility in applications, as a single plain membrane can be used for preparing more than one sensor according to the analyte in the soaking solution.

The solvent mediator (plasticizer) acts as a liquefying agent for membrane components, and it permits the mobility of the ions inside the membrane (28). Moreover, it facilitates mobility of LOM from the aqueous phase into the organic membrane, assisting the ion-exchange process. Different plasticizers were tried, such as dioctyl phthalate, dibutyl sebacate, and NPOE. The polar plasticizer, NPOE, gives the best response, and this can be attributed to the polar nature of LOM (log p 0 and –0.39). Hence, we used NPOE as a solvent mediator for LOM sensor I.

In attempt to increase sensor I sensitivity and selectivity, an ionophore (2-HP βCD) was incorporated in fabrication process of sensor II. 2-HP βCD acts as a molecular receptor (host molecule), which recognizes the structure of the guest molecule (29). Therefore, it enhances the interaction between membrane components and LOM molecules. Sensor II gave better slope, detection limit, and response time compared with those obtained by sensor I (Table 1).

**Table 1. Electrochemical response characteristics of the investigated sensor I and sensor II**

| Parameter | Sensor I | Sensor II |
|---|---|---|
| Slope, mV/decade[a] | 55.829 | 58.229 |
| Intercept, mV[a] | 263.87 | 273.17 |
| Correlation coefficient | 0.9995 | 0.9997 |
| LOD, mol/L[b] | $6.31 \times 10^{-6}$ | $5.01 \times 10^{-6}$ |
| Response time, s | 8 | 5 |
| Working pH range | 2–6 | 2–6 |
| Concentration range, mol/L | $1 \times 10^{-5}$–$1 \times 10^{-2}$ | $1 \times 10^{-5}$–$1 \times 10^{-2}$ |
| Stability, days | 30 | 30 |

[a] Average of three determinations.

[b] LOD (according to IUPAC definition: measured by intersection of the extrapolated arms of nonresponsive and the Nernstian segments of the calibration).

The electrochemical performance characteristics of sensors I and II were evaluated according to IUPAC recommendations (23). Table 1 shows the results obtained for both sensors over a period of 4 weeks. Calibration plots are shown in Figure 2. The sensors give constant potential readings within ±1 mV from day to day. The dynamic response time is important in the evaluation of ion-selective electrodes. The required time for the sensors to reach values within ±1 mV was recorded by increasing LOM concentration by 10-fold.

The effect of pH on the response of the two investigated sensors was studied. Results reveal an approximately stable potential over pH range from 2 to 6 for both sensors. At higher pH values, a gradual and then sharp decrease in the potential values is observed, which is explained by the formation of the nonprotonated LOM in alkaline medium. LOM pKa is 8.7; therefore, it is expected to be ionized below the pH of 6.7 and nonprotonated above this value. This pH range makes the sensors suitable for determining LOM in its dissolution medium (0.01 N HCl) described by the U.S. Food and Drug Administration (FDA; 30).

The effect of temperature on the response of the proposed sensors was evaluated. A slight increase in the response was detected as temperature increases in the range of 25 to 37°C. However, parallel calibration plots of relatively similar slopes at different temperatures were obtained. Furthermore, LODs and response times did not vary significantly, indicating a reasonable thermal stability of the proposed PVC sensors.

The effect of excipients, organic- and inorganic-related substances, was evaluated by the separate solutions method (23). The electrodes show a high selectively for the drug in 0.01 N HCl LOM dissolution medium described by the FDA (Table 2).

The results reveal that sensor II is better than sensor I in terms of slope, detection limit, and response time (Table 1), so it was the sensor of choice for the dissolution experiment.
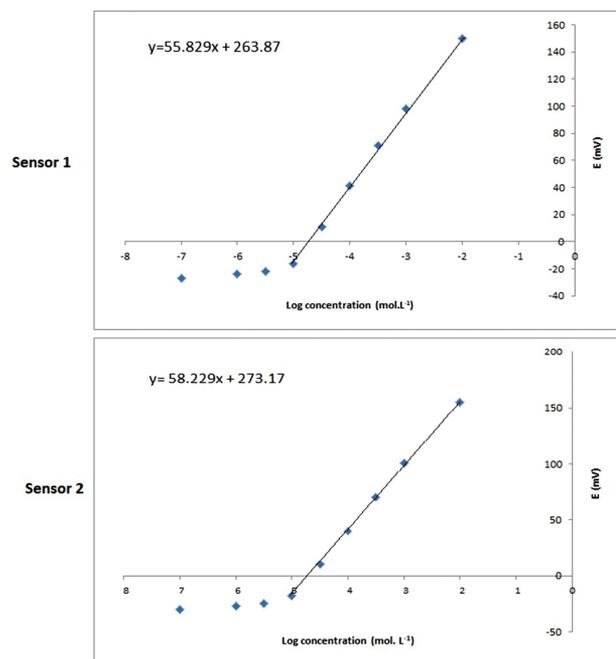
**Table 2. Potentiometric selectivity coefficients ($K^{pot}_{LOM}$) of the proposed sensors using the separate solutions method**

| Interferent, $10^{-3}$ mol/L | Sensor I | Sensor II |
|---|---|---|
| Glucose | $2.24 \times 10^{-4}$ | $2.88 \times 10^{-4}$ |
| Lactose | $1.29 \times 10^{-4}$ | $2.09 \times 10^{-4}$ |
| Urea | $2.40 \times 10^{-4}$ | $3.24 \times 10^{-4}$ |
| Talc | $3.89 \times 10^{-5}$ | $7.76 \times 10^{-5}$ |
| Starch | $5.50 \times 10^{-5}$ | $7.59 \times 10^{-5}$ |
| $(NH_4)_2SO_4$ | $4.68 \times 10^{-3}$ | $7.08 \times 10^{-3}$ |
| KCl | $1.74 \times 10^{-3}$ | $2.75 \times 10^{-3}$ |
| NaCl | $5.50 \times 10^{-3}$ | $5.89 \times 10^{-3}$ |
| $CaCl_2$ | $5.75 \times 10^{-3}$ | $5.37 \times 10^{-3}$ |

### Method Validation

The proposed method was validated according to The United States Pharmacopeia guidelines (31; Table 3).

At six different concentrations ranging from $1 \times 10^{-5}$ to $1 \times 10^{-2}$ mol/L, calibration curves were created. The range covers approximately from 0.87 up to 900% of the labeled amount (441.5 mg/tablet). A linear correlation is observed between the potential in millivolts and Log molar concentration of LOM.

The effect of excipients was tested in 0.01 N HCl as the dissolution medium of LOM, and no interference is found.

**Table 3. Assay validation sheet of the proposed sensor I and sensor II**

| Parameter | Sensor I | Sensor II |
|---|---|---|
| Accuracy[a] | 99.67% | 99.87% |
| | Precision | |
| Repeatability[b] | ±0.82 | ±0.75 |
| Intermediate precision[c] | ±1.19 | ±1.04 |
| Robustness | ±0.76[d] | ±0.73[e] |
| LOD, mol/L[f] | $6.31 \times 10^{-6}$ | $5.01 \times 10^{-6}$ |
| | Linearity | |
| Slope | 55.829 | 58.229 |
| Intercept | 263.87 | 273.17 |
| Correlation coefficient | 0.9995 | 0.9997 |
| Range, mol/L | $1 \times 10^{-5} - 1 \times 10^{-2}$ | $1 \times 10^{-5} - 1 \times 10^{-2}$ |

[a] The accuracy ($n = 5$), average of three concentrations ($10^{-5}$, $10^{-3}$, and $10^{-2}$ mol/L) of LOM.

[b] The intraday ($n = 5$) RSD of three concentrations ($10^{-5}$, $10^{-3}$, and $10^{-2}$ mol/L) of LOM repeated five times within a day.

[c] The interday ($n = 5$) RSD of three concentrations ($10^{-5}$, $10^{-3}$, and $10^{-2}$ mol/L) of LOM repeated five times in five successive days.

[d] Robustness ($n = 3$), RSD of determinations of three concentrations ($10^{-5}$, $10^{-3}$, and $10^{-2}$ mol/L) of LOM under variation of pH of the solvent (±0.2)

[e] Robustness ($n = 3$), RSD of determinations of three concentrations ($10^{-5}$, $10^{-3}$, and $10^{-2}$ mol/L) of LOM in different dissolution conditions.

[f] Limit of detection (measured by interception of the extrapolated arms of Figure 2).



**Figure 2. Profile of the potential in millivolts versus log concentrations of LOM in moles per liter obtained with sensor I and sensor II**

The accuracy is demonstrated by evaluating the recovery of three known concentrations ($10^{-5}$, $10^{-3}$, and $10^{-2}$ mol/L) of LOM in 0.01N HCl. Satisfactory results are obtained as shown in Table 3.

The precision of the method is determined by measuring the potential in millivolts of three different concentrations ($10^{-5}$, $10^{-3}$, and $10^{-2}$ mol/L) five times within the same day (repeatability) and on different 5 days (intermediate precision), and the SD was lower than 2 ($n = 5$) as shown in Table 3.

Robustness is evaluated by making small deliberate changes, such as change in pH, agitation rate, and temperature. Results presented in Table 3 indicate that the method is robust with RSD, %, less than 2%.

### Potentiometric Determination of Laboratory-Prepared Mixtures Containing Different Ratios of LOM and BZCL

Results reveal that the proposed sensors can be successfully used to determine LOM in the presence of BZCL with no need for prior separation as shown in Table 4.

### Potentiometric Determination of LOM in its Pharmaceutical Formulations

The investigated sensors were applied to determine LOM in tablets and eye drops in an aqueous solution without preliminary drug extraction or derivatization. Percent recovery was $100.34 \pm 0.59$ and $100.67 \pm 0.58$ for Lomeflox tablets and Orchacin eye drops, respectively. Results obtained by potentiometric method were statistically compared with those obtained by the reported HPLC method (8). Student's $t$-test and $F$-value were calculated, and no significant difference was observed between both methods, indicating the applicability of the new method for analysis of pharmaceutical preparations.

### Potentiometric Determination of LOM in Plasma

The proposed sensors were able to determine LOM concentration in spiked human plasma. A wide concentration range of the drug can be determined with high accuracy and precision. Results presented in Table 5 show that sensor II is more accurate and precise than sensor I.

### Potentiometric Determination of LOM in Dissolution Medium

Sensor II was able to determine LOM concentration in its dissolution medium during monitoring of LOM release from

**Table 4. Potentiometric determination of laboratory-prepared mixtures containing different ratios of LOM and BZCL**

| LOM concn, mol/L | BZCL concn, mol/L | % Rec. of LOM[a] | |
| --- | --- | --- | --- |
| | | Sensor I | Sensor II |
| $10^{-3}$ | $8.17 \times 10^{-7}$ (50%)[b] | 99.63 | 99.02 |
| $10^{-3}$ | $1.63 \times 10^{-6}$ (100%) | 99.63 | 99.02 |
| $10^{-3}$ | $3.27 \times 10^{-6}$ (200%) | 99.63 | 99.02 |
| $10^{-3}$ | $4.90 \times 10^{-6}$ (300%) | 99.63 | 100.00 |
| $10^{-3}$ | $6.53 \times 10^{-6}$ (400%) | 99.63 | 100.00 |

[a] Average of three determinations.

[b] In relation to concentration of BZCL in dosage form.

**Table 5. Determination of LOM in spiked human plasma by the proposed sensors**

| Added, M | % Recovery ± SD[a] | |
| --- | --- | --- |
| | Sensor I | Sensor II |
| $10^{-3}$ | $98.64 \pm 0.34$ | $98.94 \pm 0.33$ |
| $10^{-4}$ | $99.80 \pm 0.45$ | $99.97 \pm 0.25$ |
| $10^{-5}$ | $100.26 \pm 0.36$ | $100.12 \pm 0.20$ |

[a] Average of three determinations.

tablets. There must be accurate description for the entire concentration range during dissolution process from zero concentration at the beginning of the process to the maximum concentration released at end of the process.

At the beginning (zero LOM concentration), the conversion of the obtained potential to percent dissolution cannot be performed using Nernst equation, but it is possible by using the transpose of the following Nikolskii–Eisenman equation (24):

$$C_{analyte} = C_{st}\left(10^{E/S} - 1\right)$$

This equation converts the measured potential in millivolts to concentration values using the slope ($S$), which is obtained from the calibration curve measured prior to the dissolution test at the same conditions of the dissolution test, and a constant value called ($C_{st}$), which was calculated from the obtained potential at previously mentioned known concentrations. $C_{st}$ was obtained by inserting the electrode in solutions of known concentration of LOM at the end of the dissolution testing. An average value of $C_{st}$ was used to convert the obtained potential into concentration, and then the dissolution curve was plotted as percentage dissolution versus time (Figure 3). This equation can be applied over the entire concentration range from 0 to 100% LOM, regardless of the linearity range of the sensor (24).

### Comparison Between Potentiometric and Traditional HPLC Methods for Dissolution Monitoring

LOM is a nonofficial drug, and there are no reported methods for its dissolution monitoring. The FDA described the conditions for the dissolution testing of LOM (30). A reported HPLC
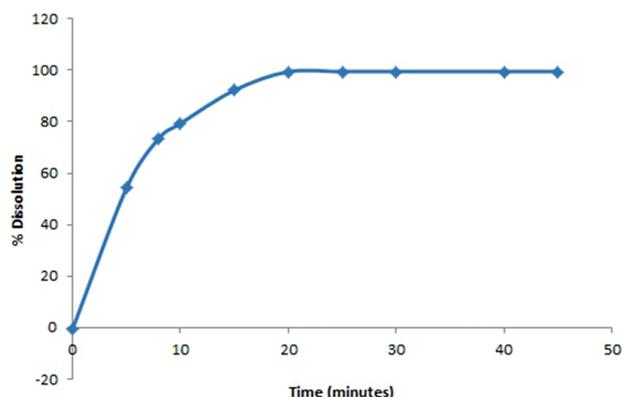
**Figure 3. Dissolution profiles for Lomeflox tablets by in-line potentiometric method.**
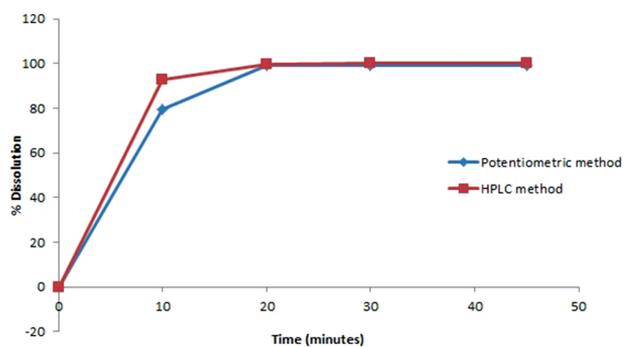
**Figure 4.   Dissolution profiles for Lomeflox tablets by in-line potentiometric and reported HPLC procedures.**

method (8) was chosen for monitoring LOM dissolution together with the potentiometric method for comparison purposes. The reported HPLC method was used to obtain the dissolution curve of LOM for Lomeflox tablets, and it was compared with the curve obtained from the suggested in-line potentiometric method (Figure 4). Figure 4 shows that the dissolution profiles do not differ significantly by applying the two methods. The similarity and difference factors were applied to compare the dissolution profiles mathematically, in which only one value is obtained to describe the closeness of the two profiles. The two factors are calculated from the following equations (32):

$$f_1 = \left\{ \sum_{t=1}^{n} |R_t - T_t| \Big/ \sum_{t=1}^{n} R_t \right\} \times 100$$

$$f_2 = 50 \log \left\{ \left( 1 + \frac{1}{n} \sum_{t=1}^{n} (R_t - T_t)^2 \right)^{-0.5} \times 100 \right\}$$

where $f_1$ is the difference factor; $f_2$ is the similarity factor; $n$ is the number of dissolution sample times; and $R_t$ and $T_t$ are the percent dissolved at each time point, $t$, for the reference and test dissolution profiles, respectively. The FDA's guidance for industry also recommends these factors for comparing dissolution profiles. According to the FDA guidelines, generally, $f_1$ values up to 15 (0–15) and $f_2$ values greater than 50 (50–100) ensure similarity or equivalence of the two curves (32). The two factors were calculated for Lomeflox tablets dissolution profiles, where $f_1$ is 3.85 and $f_2$ is 60.69. The results indicate that the profile obtained by the simple potentiometric method is similar to that obtained by the reported HPLC method.

### Greenness Assessment of the Proposed Potentiometric Method versus Reported HPLC Method

Potentiometric measurements are inexpensive, time saving, nondestructive, and green methods with no negative impact on environment compared with classical methods for dissolution monitoring, especially HPLC. The greenness of the proposed potentiometric method was evaluated by the Analytical Eco-Scale semiquantitative approach (33). The evaluation relies on assigning a number of penalty points to each step of the analysis method, subtracted from a base of 100. This approach was used to determine the greenness of the analytical method and for comparison purposes (33). The detailed total penalty points for our proposed potentiometric method and the reported HPLC method (8) are presented in Table 6. Based on

**Table 6.   Penalty points for LOM determination using the proposed potentiometric method and the reported HPLC method**

| Reagents | Proposed method Penalty points | Reported method Penalty points |
|---|---|---|
| HCl: 1 mL | 2 | 0 |
| Potassium dihydrogen orthophosphate | 0 | 0 |
| Disodium hydrogen orthophosphate | 0 | 0 |
| Acetonitrile | 0 | 6 |
| Glacial acetic acid | 0 | 2 |
| Water | 0 | 0 |
| Instruments | | |
| Digital ion analyzer | 0 | 0 |
| Magnetic stirrer | 0 | 0 |
| HPLC | 0 | 1 |
| Occupational hazard | 0 | 3 |
| Waste | 3 | 8 |
| Total penalty points | 5 | 20 |
| Analytical Eco-scale total score | 95 | 80 |

the obtained results, our potentiometric method is considered to be greener than the reported HPLC one. This outcome can be extended to all the reported methods, as they use hazardous organic solvents and produce a high amount of waste.

### Conclusions

Generally, ion-selective electrodes offer simplicity in design and low limit of detection. They are rapid, inexpensive, and universal, as they can be conditioned with any drug for its determination. The fabricated sensors are simply prepared and used to determine LOM in different formulations and in plasma without prior extraction or separation. Comparison between the green in-line potentiometric method and the reported HPLC method reveals no significant difference in percent dissolution along the dissolution process testing. The green in-line potentiometric method offers many advantages that make it a good alternative to classical reported methods for LOM determination. Now, ion-selective electrodes can compete with the many sophisticated methods currently available for charged drugs determination.

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